Large-Scale Supercritical Carbon Dioxide Extraction and Supercritical Carbon Dioxide Countercurrent Extraction of Cloudberry Seed Oil

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Dried press residue of cloudberry [*Rubus chamaemorus* (*Rosaceae*)] was extracted with carbon dioxide at pressures of 90−300 bar and at a temperature of 40 or 60 °C using a pilot-scale or a production-scale plant. The yield of the extract at the highest pressure was approximately 15% less than that obtained with Soxhlet extraction using diethyl ether as solvent. The extracts were either solids or viscous oils depending on the amount of neutral lipids, which increased with increasing pressure. No significant differences in the composition of the major constituent fatty acids in any of the extracts were found. The color of the extracts was clearly dependent on the amount of carotenes, which consisted mainly of β-carotene. The content of carotenes in the extracts did not increase at pressures higher than 150 bar. The amount of tocopherols in the extracts obtained at highest pressure was found to be approximately 3 times less than that at lower pressures. Countercurrent CO_2 extraction of the cloudberry oil extracted at 300 bar and 40 °C resulted in enrichment of tocopherols in the extracts and a decrease in the amount of carotenes. The concentrations of tocopherols and carotenes in all of the CO_2 extracts, the countercurrent extracts, and the raffinates were found to be clearly higher than those in the edible part of fresh cloudberry reported by other authors.

Keywords: *Carbon dioxide; cloudberry; countercurrent; pilot-scale; supercritical fluid extraction*

Extraction with dense CO_2 has been used for isolation of oils, for example, from soybeans (Stahl *et al.*, 1980; Friedrich *et al.*, 1982; Eggers *et al.*, 1985; Vasconcelos *et al.*, 1989), sunflower seeds (Stahl *et al.*, 1980), rapeseeds (Stahl *et al.*, 1980; Eggers *et al.*, 1985), and evening primrose seeds (Favati *et al.*, 1991). The extracts contained mostly neutral lipids with traces of phospholipids. Soxhlet extraction with *n*-hexane has been reported to yield slightly higher amounts of oil with a higher concentration of phosphorous compounds (Friedrich *et al.*, 1982), although phospholipids are insoluble also in *n*-hexane (Christie, 1982a). In the extraction of fats and oils with supercritical CO_2, many fat soluble compounds, which are poorly soluble in CO_2 as such, are often coextracted in higher amounts than would be expected based on the solubility of the pure compounds in supercritical CO_2. For example, pure carotenoids are soluble at approximately 0.03% by weight in CO_2 at 300 bar and 40 °C; however, if fats are extracted in larger amounts, the solubility of carotenoids is enhanced to 0.08% by weight (Stahl *et al.*, 1988). This is a consequence of the lipids acting as an entrainer for the carotenoids.

Some of the oils commercially produced, such as soya oil, corn oil, wheat germ oil, sunflower oil, and safflower oil, contain significant amounts of tocopherols (Piironen, 1986). Less attention has been paid to berries, such as cloudberry, as a source of tocopherol-rich oils. Cloudberry seeds, as berry seeds usually, contain only unsaturated triacylglycerols, having up to nine double bonds in the acyl chains (Johansson *et al.*, 1997). In addition, carotenoids have been reported to be present in considerably high concentrations in cloudberry (Heinonen, 1989). However, the seeds of many berries are small in size and the content of oil is too low for efficient oil pressing; therefore, supercritical CO_2 extraction of ground seeds would be a potential choice for the isolation of valuable constituents from berries.

This study demonstrates the supercritical CO_2 extraction of cloudberry with a pilot-scale and a production-scale plant. The effect of extraction conditions on the composition of lipids, carotenes, and tocopherols in the extracts is discussed. In addition, supercritical CO_2 countercurrent extraction was briefly studied for the fractionation of the cloudberry oils extracted with supercritical CO_2.

EXPERIMENTAL PROCEDURES

Materials. Frozen cloudberries [*Rubus chamaemorus* (*Rosaceae*)] received from Russia were slowly melted before removal of the juice by cold pressing at Rovaniemi Biological Research Center (Rovaniemi, Finland). The press residue (1000 kg) was stored as frozen blocks until dried at 40 °C for 6−12 h on a plat dryer (KTTK Siementarkastusosasto, Loimaa, Finland). The total weight of the dried residue was 271 kg, and the water content was 4−5%. The dried cloudberry material, of which 42 kg was used for pilot plant processing, was ground just before extractions. The grinding was conducted with a pin wheel mill cooled with liquid CO_2. The remaining ground material was extracted with a production plant. The residue from dense CO_2 extractions was further collected and stored in plastic bags at −20 °C for Soxhlet extraction.

The supercritical CO_2 extracts, countercurrent extracts and raffinates, and Soxhlet extracts were stored under nitrogen at −20 °C to avoid oxidation and diluted in *n*-hexane (HPLC grade; Rathburn Chemicals Ltd.,Walkerburn, Scotland) before liquid chromatography. The reference compounds of β-carotene and tocopherols were purchased from Sigma Chemical Co. (St. Louis, MO) and Merck (Darmstadt, Germany), respectively. The Soxhlet extracts were further fractionated into lipid classes according to the procedure introduced by Christie (1982a). The elution of the extracts through a silica gel 60 (Merck) tube with diethyl ether (analytical reagent AR containing 5 ppm BHT as stabilizer; Labscan Ltd., Dublin,

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Ireland), acetone (HPLC grade; Merck), and methanol (HPLC grade; Merck) resulted in the fractions of neutral lipids, glycolipids, and phospholipids, respectively. The amounts of the lipid fractions were measured gravimetrically. Fatty acid methyl esters for gas chromatography were prepared by sodium methoxide-catalyzed transesterification (Christie, 1982b) and purified by a Florisil (Fluka Chemie AG, Buchs, Switzerland) tube with *n*-hexane/diethyl ether solution (99:1, vol:vol).

Methods. *CO_2 Extraction.* Extractions with CO_2 at high pressures were obtained on pilot-scale and production-scale extraction plants at Flavex (Rehlingen, Germany). The volumes of extraction vessels of the pilot plant and the production plant were 2 × 18 and 3 × 150 L, respectively. The pilot plant extractions of dried cloudberry press residue (6 × 7 kg) were performed at 40 °C using pressures of 90, 100, 120, and 300 bar and at 60 °C at pressures of 150 and 300 bar. The production plant was operated at 40 °C and 300 bar. The mass flow of carbon dioxide was 50 kg/kg of material in each extraction in both plants.

Countercurrent Extraction. A portion (4 L) of cloudberry oil extracted with the production plant was further extracted with a countercurrent column system at Flavex. The height of the column was 3 m and the inner diameter 46 mm. The column was filled with stainless steel spirals to achieve 3−5 separation plates. The extractions were performed at 40 °C and 230 bar. The use of CO_2 mass flow rates of 35, 68, and 170 kg/kg of oil resulted in extract/raffinate ratios of 24/76, 40/60, and 90/10, respectively.

Soxhlet Extraction. Three samples of 6−10 g of original cloudberry press residue and each of the CO_2 extraction residues were extracted for 4 h with 180 mL of diethyl ether (Labscan Ltd.). The volume of the Soxhlet apparatus was 30 mL. The solvent was evaporated under vacuum after extraction with a rotary evaporator to dryness. The yield of oil was measured gravimetrically, after which the oil was removed into test tubes with a small amount of diethyl ether for further steps.

Gas Chromatography (GC). A Varian 3300 gas chromatograph (Varian, Palo Alto, CA) equipped with a flame ionization detector (FID, 240 °C) and a split/splitless injector (220 °C) was applied to the analysis of fatty acid methyl esters. A 20 m × 0.32 mm i.d. NB-351 column (Nordion Instrument Ltd., Helsinki, Finland) with a film thickness of 0.20 μm was used for the separation. The temperature program applied to the analysis was from 120 °C (3 min isothermal) with 3 °C/min to 210 °C (10 min isothermal) using helium as a carrier gas with a flow rate of 1.5 mL/min.

Liquid Chromatography (LC). A Shimadzu LC-9A liquid chromatograph (Shimadzu Co., Kyoto, Japan) with a 250 × 4.0 mm i.d. LiChrosorb Si 60 (5 μm) column (E. Merck, Darmstadt, Germany) was used for the analysis of β-carotene and tocopherols. A solution of *n*-hexane/2-propanol (99.0:1.0, vol:vol) was used for the elution of β-carotene; 2-propanol was purchased from Merck. Tocopherols were eluted with a solution of *n*-hexane/2-propanol (99.4:0.6, vol:vol). The sample volume injected was 10 μL, and a flow rate of 0.9 mL/min was applied for both elutions. Internal standard in both elutions was σ-tocopherol. A Shimadzu SPD-6AV UV−vis spectrophotometric detector was used for the detection of β-carotene at a wavelength of 450 nm, and a Shimadzu fluorescence HPLC monitor RF-530 with an extinction wavelength of 290 nm and an emission wavelength of 330 nm was used for the detection of tocopherols.

RESULTS AND DISCUSSION

CO_2 Extraction. The total yields of oil from CO_2 extractions and the oil obtained from the Soxhlet extraction of the CO_2 extraction residue with diethyl ether were in good agreement with that obtained with Soxhlet extraction from the original dried cloudberry material (Figure 1). At the highest CO_2 extraction pressure (300 bar), the pilot plant yield of oil was approximately 15% less than that obtained with Soxhlet extraction of the original dried cloudberry material.

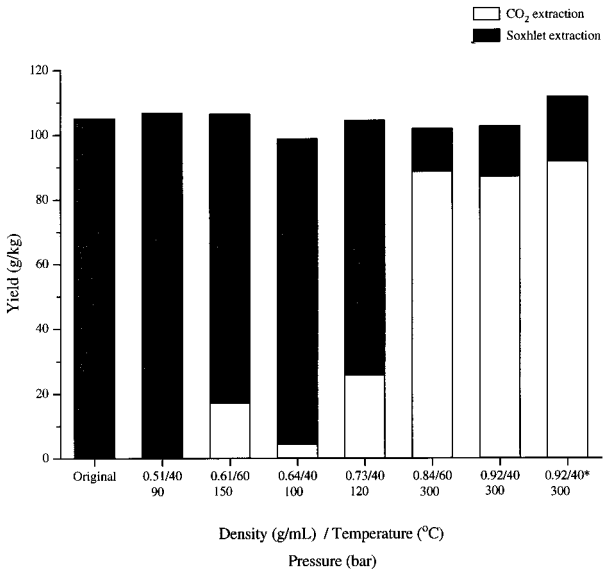

Figure 1. Yield of oil from the cloudberry press residue, achieved by CO_2 extraction, and from the CO_2 extraction residues, achieved by Soxhlet extraction (*production plant). The sum of the two yields in each column represents the total oil content in the original cloudberry press residue.

Fractionation of the oil from the Soxhlet extraction of the original dried cloudberry material resulted in 93.0% yield of neutral lipids, 3.8% of glycolipids, and 3.2% of phospholipids by weight, whereas the corresponding proportions of the fractions in the oil of the residue from the pilot plant CO_2 extraction at 300 bar and 40 °C were 70.3, 23.5, and 6.2 wt %, respectively. Thus, approximately 11% of the total neutral lipids in the cloudberry material remained unextracted corresponding to approximately 1% of oil in the press residue. In the case of residue from the production plant operated at the same extraction conditions, the amount of unextracted neutral lipids was slightly higher. At the lowest extraction pressure, the yield of extract was too low to be collected quantitatively and, therefore, was not further analyzed.

The amount of phospholipids in CO_2 extraction residues decreased from 3 to 1 mg/g with the increasing CO_2 extraction pressure, as the amount in the original press residue was found to be 3 mg/g. The amount of glycolipids was approximately the same in the original press residue and in each CO_2 extraction residue (3 mg/g). This indicated that the coextraction of phospholipids was slightly enhanced at higher pressures, whereas glycolipids were extracted only as trace amounts, if any. The very poor solubility of glycolipids in the system could be explained with the high number of free hydroxyl groups in the sugar moiety of glycolipids. The difference in the yield of oil in CO_2 extraction and in Soxhlet extraction of the original press residue was mainly due to the difference in the solubility of phospholipids in extraction media. This kind of phenomenon has also been reported by Friedrich *et al.* when they compared the yield of lipids in CO_2 extraction and Soxhlet extraction with *n*-hexane of soybeans (Friedrich *et al.*, 1982).

The effect of temperature on the supercritical CO_2 extraction is seen by comparing the yields of extract at 0.64 g/mL/40 °C and 0.61 g/mL/60 °C (Figure 1). The yield of extract was almost 4-fold greater at 60 °C. This could be explained as an effect of an increased diffusion of the lipids from the matrix to supercritical extraction medium. The vapor pressure of lipids is negligible in

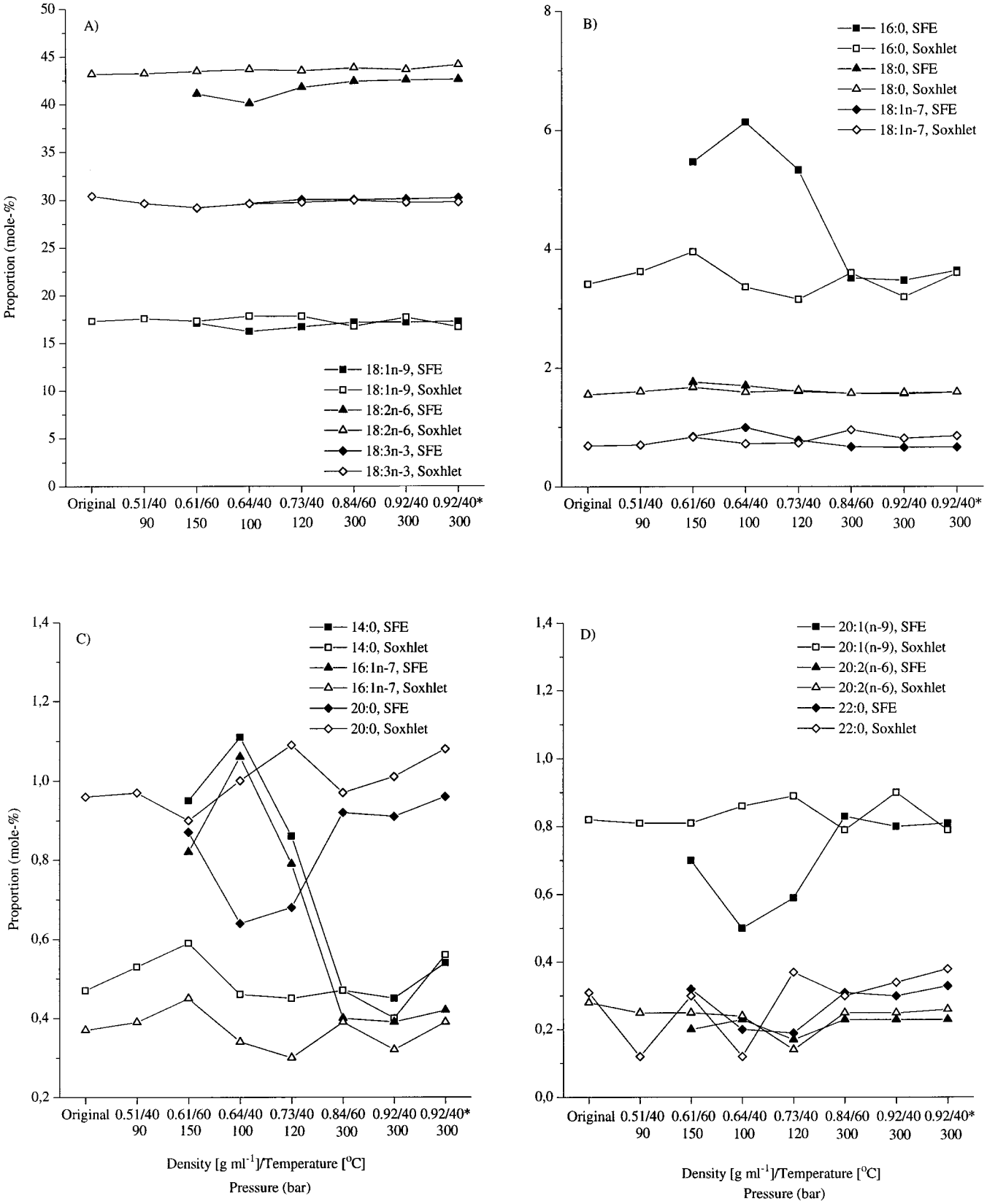

Figure 2. Proportion of fatty acids in the extracts of CO_2 and Soxhlet extraction (*production plant; SFE, oil obtained from the cloudberry press residue by CO_2 extraction; Soxhlet, oil obtained from the CO_2 extraction residues by Soxhlet extraction).

comparison to the extraction pressure, since the boiling point of lipids is above 200 °C. Therefore, the increase in temperature by 20 °C has no effect on the volatility of the lipids. Although the respective densities were approximately the same in both cases, the pressures were $p_{0.64/40} \approx 100$ bar and $p_{0.61/60} \approx 150$ bar. It is more reasonable to compare densities than pressures at different temperatures, since the solvent power of supercritical fluid is to first approximation related to density. The effect of temperature on the yield of oil can also be seen at 300 bar using temperatures of 40 °C ($\rho \approx 0.92$ g/mL) and 60 °C ($\rho \approx 0.84$ g/mL).

The most abundant fatty acids in cloudberry seed oil extracts were oleic acid (18:1$n-9$), linoleic acid (18:2$n-$

6), and α-linolenic acid (18:3*n*−3). No distinctive differences were found in the proportions of these fatty acids in any of the oils (Figure 2). This was in good agreement with other reports (Stahl *et al.*, 1980; Favati *et al.*, 1991; Maness *et al.*, 1995). Fractionation of triacylglycerols according to acyl carbon number was not expected, because the acyl carbon number range of cloudberry triacylglycerols is very narrow (ACN = 48−56) (Manninen *et al.*, 1995). Some alterations of the molar proportions, however, were observed in the case of less abundant fatty acids; for example, the proportion of 16:0, 14:0, and 16:1*n*−7 was the lowest in the oils extracted at the highest pressure. In addition, the proportion of 20:0 and 20:1*n*−9 increased with increasing pressure. These changes in the molar compositions of fatty acids led to the assumption that some fractionation of triacylglycerols occurred, although this appears to have no practical value.

The presence of carotenoids in the oils and in the CO_2 extraction residues was studied using LC−UV at a detection wavelength of 325 nm. The chromatograms showed only trace amounts of carotenoids other than β-carotene. This result was expected, since the conjugated double-bond system of the β-carotene already results in limited solute solubility in CO_2. Any additional polar functional group on the carotenoid molecular structure effectively decreases the solubility of that species. The quantitation of α+β-carotene was performed at a detection wavelength of 450 nm to minimize the possible disturbances in detection by other sample components. The LC method did not distinquish α- and β-carotene or their *cis*- and *trans*-isomers; however, this did not affect the results, since the response factor of α- and β-carotene was considered to be the same.

Figure 3 shows the yield of α+β-carotene in the CO_2 and Soxhlet extractions and the amount of α+β-carotene in the CO_2 and Soxhlet extracts. As the yield of α+β-carotene (Figure 3a) is compared with the yield of extracts (Figure 1) in the CO_2 extraction, it can be concluded that the higher yield of carotenes was more likely a result of their enhanced coextraction with lipids, rather than due to the enhanced solubility in CO_2. This phenomenon can also be seen in Soxhlet extraction by comparing the yield of α+β-carotene and the yield of extracts: as the amount of lipids was decreasing in the CO_2 extraction residues, the yield of α+β-carotene was decreasing, since carotenes have similar solubility in diethyl ether and in supercritical CO_2. The amount of α+β-carotene in the oil extracted at a density of 0.92 g/mL (300 bar) and 40 °C with the production plant was 0.64 mg/g (Figure 3b), which corresponds to the amount of 58.7 μg/g in the cloudberry press residue. The sum of the amount of α- and β-carotene in the edible part of cloudberry, which does not include the seeds, has been reported to be 2 μg/g in fresh weight (Heinonen, 1989).

The proportion of α- and γ-tocopherol both in the CO_2 extracts and in the CO_2 extraction residues was found to be in average 97% of the total tocopherols. The extraction behavior of tocopherols was quite similar to that of α+β-carotene (Figure 4a) with the execption of having the yield maximum at slightly lower density. The concentration of the tocopherols in the extracts was at least 3 times less at extraction densities corresponding to the pressure of 300 bar than that at lower densities as can be seen from the vitamin E equivalent values in Figure 4b. This was mainly due to the increase in the proportion of lipids in the extracts as the density and/

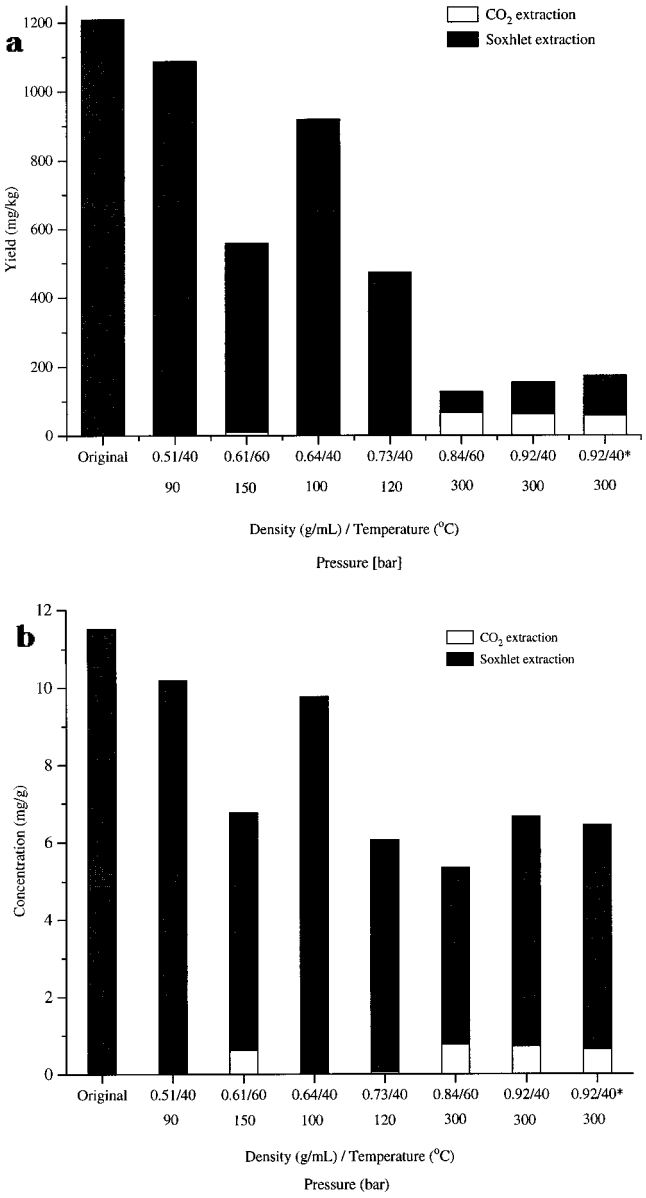

Figure 3. (a) Yield of α+β-carotene in the extract from cloudberry press residue achieved by CO_2 extraction and in the extract from CO_2 extraction residue achieved by Soxhlet extraction. (b) Concentration of α+β-carotene in the corresponding extracts (*production plant).

or the temperature was increasing. The sums of the yields of tocopherols as vitamin E equivalents in the CO_2 extracts and in the corresponding CO_2 extraction residues differed significantly from the vitamin E equivalent value in the original cloudberry material. The difference of the determined values was probably not due to the storage of the CO_2 extraction residues, since the values were similar in the original material and in the residue of extraction at lowest density. It seemed evident that the loss occurred during the CO_2 extraction process or during the following steps. In principle, the CO_2 extraction process protects against oxygen. The adverse effect may be caused by traces of elements in the plant, which could act as pro-oxidants. The more obvious reason was that tocopherols were protecting the lipids against oxidation during the removal of the residual carbon dioxide from the oils, which was accomplished *via* heating at 50 °C. The vitamin E equivalent in the edible part of cloudberry has been reported to be 31 μg/g in fresh weight (Piironen, 1986). The vitamin E equivalent in the oil extracted with the

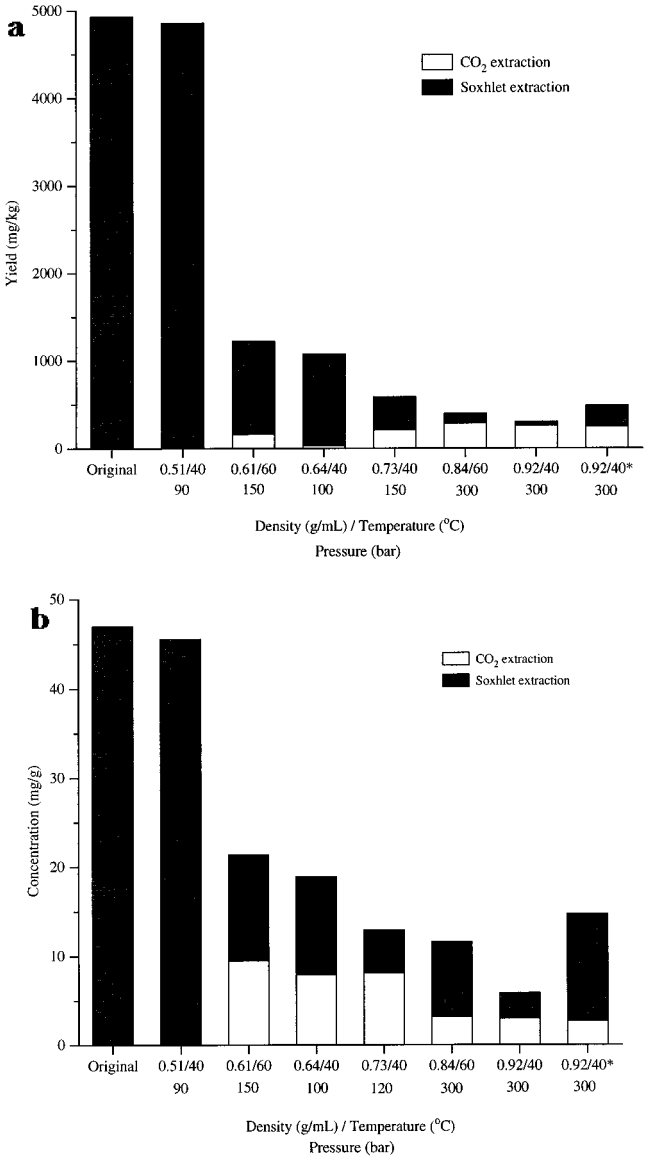

Figure 4. (a) Yield of tocopherols as vitamin E equivalent in the extract from cloudberry press residue achieved by CO_2 extraction and in the extract from CO_2 extraction residue achieved by Soxhlet extraction. (b) Vitamin E equivalent of the corresponding extracts (*production plant).

Table 1. Color and Fluidity of Cloudberry Extracts Obtained by Supercritical CO_2 Extraction

density (g/mL)/ temperature (°C)	color	fluidity
0.51/40	dark violet	solid
0.61/60	pale reddish brown	solid[b]
0.64/40	dark brown	solid[c]
0.73/40	light brown	solid[b]
0.84/60	brownish red, cloudy	viscous oil
0.92/40	brownish red, cloudy	viscous oil
0.92/40[a]	orange red, cloudy	viscous oil

[a] Production plant. [b] Melting at temperatures over 45 °C. [c] Melting at temperatures over 50 °C.

production plant was found to be 2.7 mg/g corresponding to approximately 29 μg/g of the press residue.

Each of the extracts had the characteristic odor of cloudberry. They were either solids or viscous oils, depending on the proportion of triacylglycerols in the extracts (Table 1). The proportion of triacylglycerols was found to increase from 60% to 96% as the extraction pressure increased, as measured as total FID response of the extract using a supercritical fluid chromatographic (SFC) method described elsewhere (Manninen *et al.*, 1995). The color of the extracts changed from brown to brownish red or orange red as the amount of carotenes increased. The residues from the CO_2 extractions of cloudberry press residue still contained valuable constituents, as the results of Soxhlet extraction showed, and could be further utilized, for example as a food ingredient.

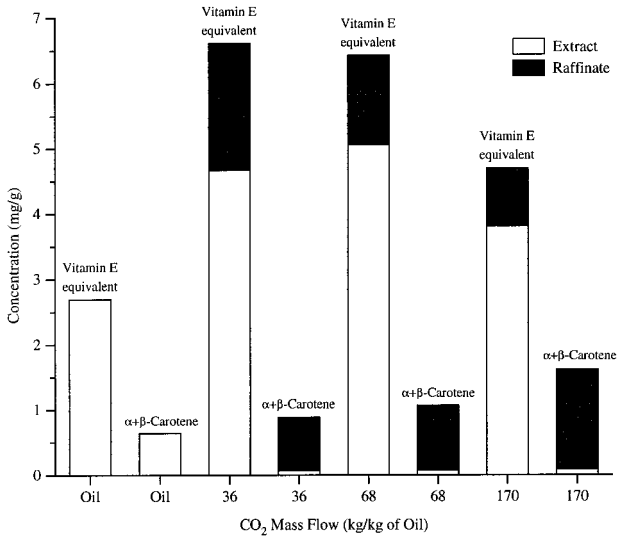

Figure 5. Concentration of $\alpha+\beta$-carotene and vitamin E equivalent in the countercurrent extracts and raffinates.

Table 2. Color and Fluidity of Cloudberry Countercurrent Extracts and Raffinates

extraction	color	fluidity
extract 1	yellowish orange	paste[a]
raffinate 1	brownish red, cloudy	oil
extract 2	yellowish orange	semipaste[b]
raffinate 2	brownish red, cloudy	oil
extract 3	orange, cloudy	semiviscous[b]
raffinate 3	brownish red, cloudy	oil

[a] Viscous oil at temperatures over 40 °C. [b] Viscous oil at temperatures over 35 °C.

Countercurrent CO_2 Extractions. A proportion of the oil obtained on CO_2 extraction with the production plant (300 bar, 40 °C) was used for countercurrent extraction experiments. No significant differences in the fatty acid composition were found in any extracts or raffinates as compared with the original oil. The available countercurrent system had only a few separation plates, and therefore, no clear fractionation of triacylglycerols was expected. The extracts had a stronger and sweeter odor of cloudberry than the original oil, whereas raffinates had no odor of cloudberry. The extracts were more like oily pastes than oils, which was due to the decrease of the proportion of triacylglycerols.

The enrichment of carotenes in raffinates was obviously due to their poor solubility in pure CO_2 (Figure 5). The content of $\alpha+\beta$-carotene in the extracts decreased approximately with a factor of 8 as compared with the original oil. This resulted in a color change from brownish red to bright orange (Table 2). The behavior of tocopherols was quite opposite to that exhibited by the carotenes. The enrichment factor in the extracts calculated as a vitamin E equivalent was, however, only 1.7 on the average (Figure 5).

ABBREVIATIONS USED

ACN, acyl carbon number; FID, flame ionization detector; GC, gas chromatography; HPLC, high-perfor-

mance liquid chromatography; LC, liquid chromatography; SFC, supercritical fluid chromatography; SFE, supercritical fluid extraction; UV, ultraviolet.

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